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Building filaments in the air: aerial morphogenesis in bacteria and fungi

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To disperse their spores to new sites, filamentous fungi and bacteria need to erect aerial filaments, which develop into fruiting bodies and spore-bearing structures. The first challenge to aerial development is breaking surface tension at an aqueous–air interface, and in both groups of microorganisms, surface-active proteins take part in the initiation of aerial morphogenesis. Comparative analysis of fungi and bacteria is providing new insights into the means by which aerial filamentation is accomplished.

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Introduction

The ability to form aerial filaments has evolved in three groups of evolutionarily distinct microorganisms: the fungi, the oomycetes, and the filamentous bacteria. These organisms normally colonise moist substrates, such as soil and decaying plant material, and grow through hyphal tip extension. The individual filaments formed by these organisms become branched and develop into a network of interwoven filaments referred to as a substrate or feeding mycelium. Conditions of stress, or encounters with hydrophobic surfaces, stimulate the raising of aerial hyphae, which then develop into reproductive structures in the form of spore chains or fruiting bodies. In filamentous fungi, reproductive structures can become very elaborate, forming complex tissues such as mushrooms and polypores, through further cellular differentiation. The erection of aerial structures is achieved through the activity of secreted proteins that allow hyphal filaments to break surface tension at the aqueous–air interface, and modulate the surface of hyphae to create a hydrophobic sheath capable of resisting desiccation [1,2].

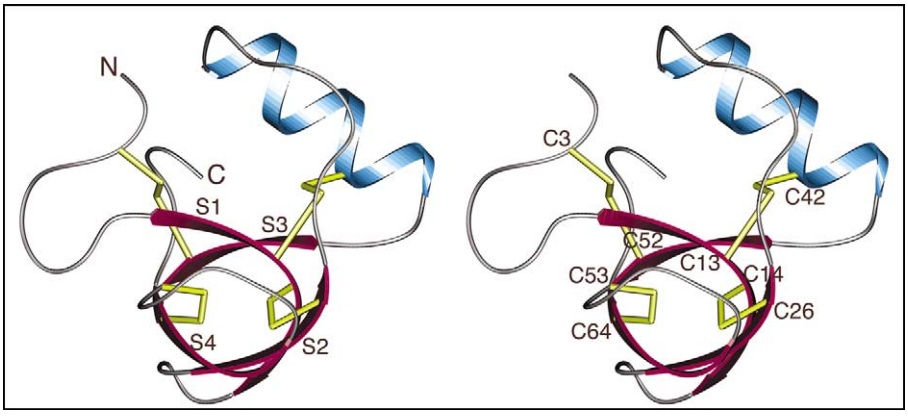
In this review, we focus on the morphogenetic proteins essential for aerial hyphal growth in both fungi and bacteria, and highlight recent advances that have contributed significantly to our understanding of aerial development.

Fungal hydrophobins — self-assembling amphiphiles

Hydrophobins are small, hydrophobic proteins that are produced by fungi during hyphal growth and development [2,3]. They play important roles in aerial hyphae formation, spore production and dispersal, stabilisation of fruiting body structures, and the virulence of some pathogenic fungi [2]. The contributions made by the hydrophobins to these diverse processes can be attributed to their unusual surface activity. They act as natural surfactants, lowering surface-tension at air–water interfaces and altering the wettability of surfaces to which they adsorb. They are secreted as monomers, but aggregate spontaneously when they encounter interfaces or surfaces. These surface-active properties of the hydrophobins allow fungi to overcome barriers formed by air–water interfaces, and permit the formation of a protective coating on the surface of the hyphae, thus preventing hyphal dehydration [1,2]. They are also involved in attachment of fungal pathogens to the hydrophobic surfaces of plants or animals, thereby contributing to pathogenicity [3,4].

Hydrophobins share limited amino acid sequence similarity, and are defined by a conserved arrangement of eight cysteine (Cys) residues. Two sub-classes of hydrophobins have been identified. In addition to having distinct biochemical properties, these two groups can be distinguished by the spacing of their cysteine residues and their characteristic distribution of hydrophobic residues [2,3]. Structural information about the hydrophobins has been difficult to obtain owing to the unusual surface-active properties of these proteins. Despite being secreted as monomers, the hydrophobins readily assemble into detergent-insoluble aggregates, and these can only be disrupted by treatment with harsh chemicals such as trifluoracetic acid, which disrupts the short-range hydrophobic interactions that contribute to aggregation. A recent breakthrough by Hakanpää *et al.* [5••] has resulted in the crystallisation, and structural determination, of a fungal hydrophobin, the HFBII class II hydrophobin from *Trichoderma reesei*. The structure reveals a novel fold, consisting of one α-helix and four anti-parallel β-sheets (forming a β-barrel) built around four intramolecular disulfide bridges. The observed disulfide linkages differ from those previously reported, and connect Cys1

Figure 1



Structure of the class II hydrophobin HFBII shown in stereo to reveal the β-barrel (blue) and four symmetrically arranged disulfide bridges (yellow). Hydrophobins have a more compact, globular structure than had been anticipated and derive their amphipathic nature from exposure of a hydrophobic patch on the surface of the molecule. Reproduced with publisher's permission from [5••].

with Cys6; Cys2 with Cys5; Cys3 with Cys4; and Cys7 with Cys8. The structure, shown in Figure 1, reveals that the four disulfide bridges are arranged symmetrically in the same plane, with two of them located entirely within the β-barrel and the other two found outside. The hydrophobin structure is therefore very stable and compact. An interesting feature revealed by the structure is a hydrophobic surface patch formed by the exposure of hydrophobic residues present in two β-hairpins. This hydrophobic patch occupies 400 $Å^2$ (12%) of the total 3200 $Å^2$ surface area of HFBII and is likely to be responsible for conferring the characteristic amphipathic properties that permit hydrophobin aggregation [5••]. Hydrophobin assembly results in formation of amyloid-like fibrils capable of reacting with dyes such as Congo Red, and these are predicted to adopt a largely unstructured β-sheet conformation [6,7]. How a compact amphiphilic, single-domain protein can assemble into such an aggregate is not yet clear, but the HFBII structure should allow for a detailed structure-function study to be conducted, examining, for instance, the role of the surface hydrophobic patch in self-assembly using targeted mutagenesis.

New functions for hydrophobins as determinants of morphogenesis

Analysis of complete genome sequences and large expressed sequence tag (EST) sets from diverse fungal species has revealed the presence of multiple hydrophobins in most fungi. For example, the tomato leaf mould fungus, *Cladosporium fulvum,* has at least six different hydrophobins (HCf-1 to 6) expressed at specific stages of development [8•]. The class I HCf-1 hydrophobin, for instance, is located on the surface of aerial hyphae and conidia, while the class II HCf-6 hydrophobin is secreted during invasive growth in plant tissue. Similarly, the corn pathogen *Fusarium verticillioides* has at least five hydrophobins (Hyd1–5), which play a role in determining the spatial patterning of spore production [9••]. Hyd1 and Hyd2, both class I hydrophobins, are required for the formation of microconidial chains, and in their absence, spores instead cluster into false heads at the end of conidiophores. In the rice blast fungus *Magnaporthe grisea*, which has six putative hydrophobin genes, aberrant expression of the *MPG1* hydrophobin in the morphological mutant *acropetal*, results in chains of conidia forming in place of the normal whorls of sympodially arrayed spores [10]. A *Δmpg1* mutant does not sporulate or form infection structures efficiently and is also unable to elaborate complete whorls of spores [4], highlighting a role for hydrophobins as morphological determinants, and not simply hydrophobic coat proteins. Further to this, the observation that spores of the pulmonary pathogen *Aspergillus fumigatus* lacking the RodA hydrophobin are more susceptible to alveolar macrophages [11•] emphasizes the variety of roles played by hydrophobins in pathogenicity as well as in development. The discovery of multi-domain hydrophobins, such as the penta-hydrophobin from *Claviceps purpurea*, further underscores the diversity of hydrophobin interaction and activity [12•].

Repellents and alternative routes to aerial morphogenesis by fungi

Many mutants lacking hydrophobins are capable of producing aerial hyphae, suggesting that additional factors must be involved in the raising of aerial structures. A family of small peptides, called repellents, has been implicated in the aerial development of the corn smut pathogen *Ustilago maydis*, a basidiomycete fungus, which is filamentous only after mating and forming a dikaryon [13]. Repellents are small peptides formed by Kex2-dependent proteolysis of a single gene product encoded by the *rep1* gene [13]. Mutants lacking *rep1* are deficient in aerial hyphae development and have reduced filament

surface hydrophobicity. This is in marked contrast to mutants lacking the two hydrophobin genes found in *U. maydis*, which were unaffected in aerial filament formation [14]. A recent study of the gill mushroom, *Schizophyllum commune*, provides further evidence that hydrophobin-independent aerial growth does occur in fungi [15•]. An abundant secreted protein, Sc15, which does not contain disulfide linkages and is unlike the hydrophobins, is found on the surface of hyphae and within mucilage. Sc15 is required for aerial hyphae formation in the absence of the Sc3 hydrophobin. The two proteins may interact at the hyphal surface, or alternatively, provide stage-specific independent mechanisms for erection of aerial hyphae [15•].

Growing aerial filaments in the filamentous bacteria

Our understanding of the morphogenetic factors necessary for aerial hyphae formation in the filamentous bacterium *Streptomyces coelicolor* has increased dramatically over the past two years, thanks to several key discoveries. It now appears that three separate groups of proteins (the rodlins, the chaplins and SapB) perform roles analogous to those of the fungal hydrophobins/repellents; however, fundamental differences between the two systems are also becoming apparent. The surface of aerial hyphae and spores in many *Streptomyces* sp. is decorated with a distinctive rodlet pattern, consisting of parallel rods 8–10 nm wide. Two highly similar proteins termed the rodlins (RdlA and RdlB) are involved in the formation of this 'rodlet layer'. These proteins are encoded by divergent genes (*rdlA* and *rdlB*), which are expressed solely during aerial hyphae formation. The rodlin genes have been identified in a variety of *Streptomyces* species [16••,17•]; however, they are missing from the recently sequenced genome of *S. avermitilis* [18]. As *S. avermitilis* sporulates efficiently, it is perhaps not surprising that deletion of the rodlin genes from *S. coelicolor* has no obvious developmental consequences. The rodlin mutant retains the ability to raise hydrophobic aerial hyphae and form hydrophobic spore chains, although the characteristic rodlet ultrastructure (not present in *S. avermitilis*) is absent from the surface of the aerial structures, and instead, a lattice of smaller fibrils (4–6 nm wide) is observed. This is in contrast to the situation in fungi, where hydrophobins that confer the rodlet pattern to the spores are also responsible for conferring hydrophobicity, and their absence results in an easily wettable phenotype [19]. Rodlin mutants do, however, show a reduced ability to attach to hydrophobic surfaces, a characteristic shared with several hydrophobin mutants [4,20].

Chaplins, determinants of surface hydrophobicity and aerial development in *Streptomyces coelicolor*

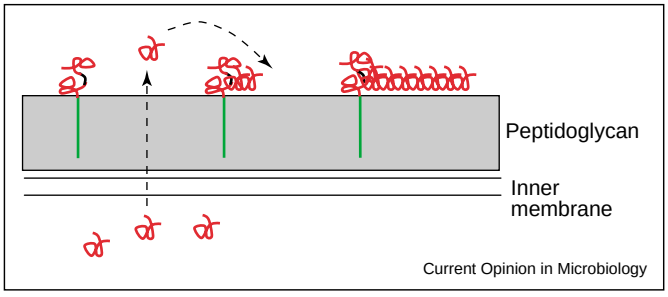
As the loss of the rodlins does not affect the hydrophobicity of the aerial hyphae and spores, the implication is that there must be additional factor(s) involved in surface modification. Recent studies have identified such a family of secreted proteins, the chaplins (coelicolor hydrophobic aerial proteins), that play a vital role in surface hydrophobicity and, consequently, in development of aerial hyphae [21••,22••]. There are eight chaplin proteins (ChpA–H) in *S. coelicolor*, all sharing a highly conserved, hydrophobic domain of ~40 amino acids, termed the 'chaplin domain'. ChpD–H are short proteins made up of a single chaplin domain, while ChpA–C are much longer, having two amino terminal chaplin domains, and a carboxy terminal 'sorting signal' that targets them for covalent attachment to the cell wall by a sortase enzyme(s) (sortases are transpeptidases that recognize a specific carboxy terminal motif, cleave the protein at that motif, and mediate attachment of the protein to a peptidoglycan cross-bridge; [23]). The chaplin genes have been identified only in sporulating actinomycetes such as *S. coelicolor*, *S. avermitilis* and *Thermobifida fusca*, and have not been observed in non-sporulating actinomycetes such as *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*. While the number of chaplin genes is variable in different organisms, the co-existence of genes for both long and short chaplins appears to be immutable.

The loss of as few as four of the eight chaplin genes (two long and two short) results in delays in aerial hyphae formation and sporulation, while deletion of all eight results in an extremely severe defect in aerial hyphae formation ([17•]; MA Elliot and MJ Buttner, unpublished) and concomitant loss of surface hydrophobicity. The observed developmental defects of chaplin mutant strains correlate well with genetic profiling experiments, which reveal that chaplin gene expression occurs at times appropriate for a role in aerial hyphae development. Additionally, chaplin gene transcripts were virtually undetectable in *bld* mutant strains [22••]. *bld* mutants are a class of developmental mutants that are defined by their inability to raise aerial hyphae, having colonies with a 'bald' appearance, rather than the fuzzy white appearance of the wild type. The majority of *bld* genes characterised thus far code for regulatory proteins [24–28].

Localisation and surface-activity of chaplins

The chaplins, like the rodlins, are all predicted to be localized to the surface of the aerial hyphae: the long chaplins through the covalent attachment of their carboxy termini to the cell wall, and the short chaplins, possibly through the heteropolymerisation of long chaplin domains with those of the short chaplins (Figure 2). All five short chaplins have been isolated from cell wall extracts, and identified using MALDI-ToF mass spectrometry. The co-localisation of the chaplins and the rodlins to the cell walls of aerial structures suggests a possible interaction between these two groups of proteins. Preliminary work by Claessen *et al.* [17•] suggests that both the rodlins and the chaplins are required for the formation

Figure 2



Localisation of the chaplins. The long chaplins are covalently anchored to the cell wall at their C-terminus. A peptidoglycan-spanning domain is shown in green, and the two chaplin domains are in red. The short chaplins consist of a single chaplin domain and are thought to be anchored to the cell wall by the long chaplins.

of the paired rodlet ultrastructure visible on the surface of spores; however, the contribution made by each to the formation of these structures is unknown. Biophysical analyses have revealed the chaplins to be rich in β-sheet secondary structure and have the ability to self-assemble into amyloid-like fibres [21••], an ability shared with the hydrophobins [6,7]. It is not known whether the rodlins have similar characteristics. The chaplins also appear to be very surface-active, and are capable of reducing surface tension from 72 to 26 mJ/m^2 [21••], an ability that is likely to facilitate the raising of hyphae from an aqueous environment into the air.

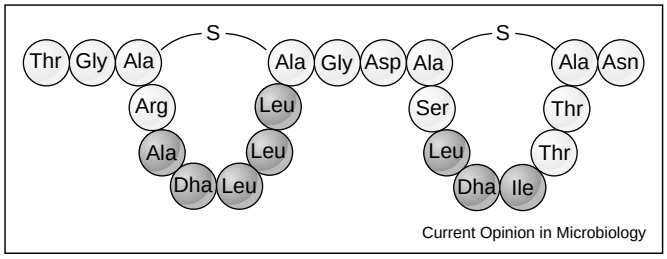
The role of SapB in aerial morphogenesis

Intriguingly, while the chaplins are necessary for the raising of aerial hyphae and conferring surface hydrophobicity to aerial structures, they alone are not sufficient for this to occur. Exogenous application of short chaplin preparations to mutants lacking multiple chaplin genes restores the formation of aerial hyphae to mutant strains [21••,22••]; however, the same preparations do not restore aerial hyphae formation to *bld* mutants [21••]. The defects in the aerial hyphae formation of *bld* mutants can however, be relieved by the exogenous application of another protein, SapB (spore associated protein B; [29]). SapB is a small hydrophobic peptide and, like the chaplins, has surfactant properties: it is able to reduce the surface tension at an air–water interface from 72 mJ/m^2 to 32 mJ/m^2 [30]. Although it was originally isolated from the surface of spores, SapB is present in considerably greater abundance in the culture media (J Willey, personal communication), in contrast to the chaplins, which are primarily localized to the aerial structures (only two of the chaplins have ever been identified in culture media). Curiously, the production of SapB appears to be media-dependent, as it is produced on rich media but not on minimal media [31].

The genes specifying SapB have been elusive for many years, but a very recent breakthrough has identified SapB as the processed product of the *ramS* gene. *ramS* is part of the *ram* (rapid aerial mycelium) gene cluster [32], which consists of the *ramCSAB* operon and the divergently expressed *ramR*. The orthologous cluster in *S. griseus* has been termed the *amf* (aerial mycelium formation) cluster [33]. Overexpression of the *ram* cluster accelerates the formation of aerial hyphae in wild-type strains, and deletion of *ramR*, *ramC* or *ramS* results in a reduced ability to raise aerial structures. RamR is a response regulator-like protein that controls expression of the *ramCSAB* operon [34•–36•]. The predicted product of *ramC* has homology to serine/threonine kinases in its amino terminal domain [37], *ramS* is predicted to encode a 42 amino acid peptide, and *ramA* and *ramB* encode components of an ABC transporter.

Since the characterisation of the *ram* gene cluster, there has been intense speculation about the possibility of *ramS* encoding SapB, but addressing this issue experimentally has been hampered by the resistance of SapB to biochemical analysis. The first step towards resolving this debate came when renewed attempts to obtain amino terminal sequence for SapB yielded five residues bearing a match to RamS beginning at residue 22, and extending to residue 26 [38••]. This coincided with the realisation that the previously uncharacterised carboxy terminal domain of RamC bore similarity to proteins involved in the maturation/processing of lantibiotic (lanthionine-containing antibiotic) peptides. Lantibiotics are ribosomally synthesised oligopeptide antibiotics that are translated as inactive pre-proteins, and are subject to extensive post-translational modification and cleavage to generate a biologically active peptide [39]. RamS is a reasonable candidate for RamC-mediated post-translational modification on the basis of its small size (42 amino acids), and the fact that it contains several residues in its carboxy terminus that would be good substrates for the introduction of lantibiotic-like modifications, in particular, five serine and two cysteine residues: serine can be dehydrated to give didehydroalanine (Dha), which can then

Figure 3



Predicted primary structure for SapB. This 21 amino acid lantiobiotic-like peptide has two hydrophobic loops (hydrophobic residues are shaded dark grey) formed by lanthionine bridges between Dha and Cys residues. This amphiphilic structure is likely to be important for the surfactant activity of SapB.

react with the sulfhydryl groups of cysteine residues to form lanthionine (Lan) bridges. It is therefore possible that RamS is subject to modification by RamC, and is cleaved between residues 21 and 22 to yield SapB. Interestingly, the cleavage of RamS at this site would result in a peptide having a mass 72 Da greater than that of SapB, and this mass difference could be accounted for by the dehydration of four serine residues (to Dha) in RamS, an essential step in the formation of Lan bridges. To examine whether SapB could be a RamS-derived lanthionine-containing peptide, it was treated with compounds that modify Dha residues and Lan bridges [38••]. This treatment permitted the determination of 11 from 21 residues of SapB via Edman degradation, and these aligned perfectly with sequences in the carboxy terminus of RamS. Additional biochemical experiments have identified the most likely positions for the modified amino acids (Dha residues) and the lanthionine bridges, and the proposed structure for SapB is shown in Figure 3 [38••]. Work carried out in parallel on AmfS in *S. griseus*, suggests that a similar structure is formed, and that the processing and modification of the AmfS peptide is required for its morphogenetic activity ([40]; K Ueda, personal communication). Modelling of the SapB structure suggests it is amphiphilic in nature, a characteristic important for activity as a surfactant and one that has parallels to the compact, amphiphilic HFBII hydrophobin structure recently reported [5••].

Genetic regulation of SapB

The discovery that SapB is specified by the *ram* gene cluster ties together and explains many disparate observations made in past years. It has long been known that *bld* mutant strains regain their ability to raise aerial hyphae upon exogenous application of SapB [41]; overexpression of *ramR* in *bld* mutant strains also restores aerial hyphae formation, and enhances SapB biosynthesis in these mutant strains [34•]. An effect is seen also in wild type strains, where the overexpression of *ramR* results in increased SapB production and accelerated development [34•,35•]. SapB is not produced when strains are grown on minimal media [31], and the *ram* genes are not transcribed during growth on minimal media [35•]. These observations are all consistent with the fact that RamR controls the expression of the SapB biosynthetic cluster (*ramCSAB*) on rich media.

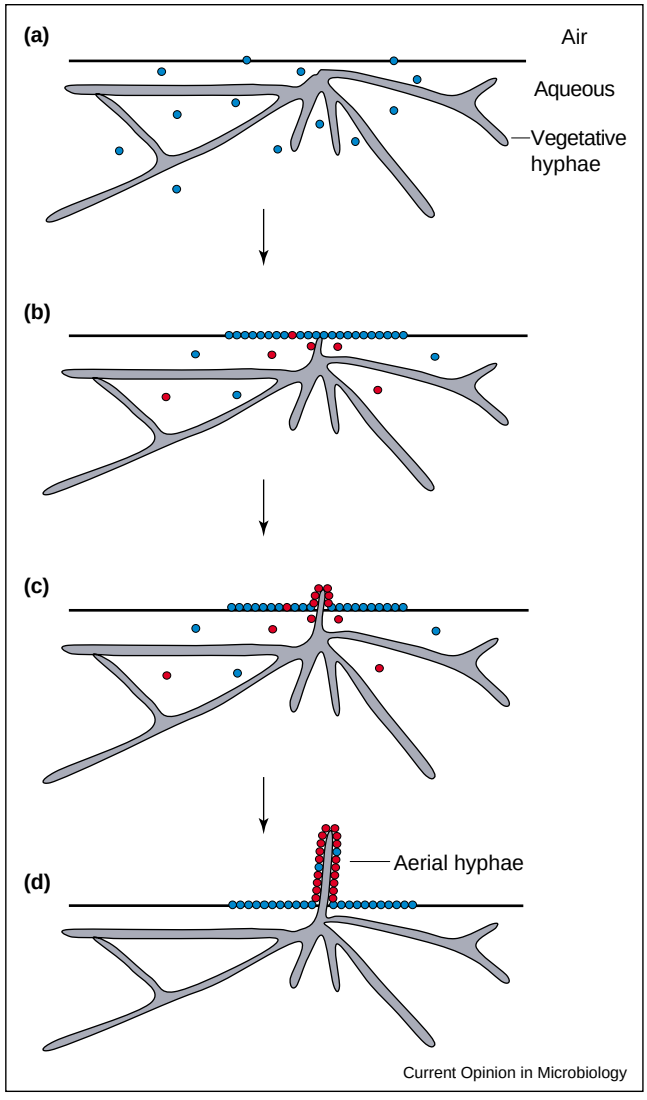
Transcription of *ramR* is observed during substrate hyphal growth, and it increases to maximal levels at the onset of aerial hyphae formation [35•]. The *ramC* transcript follows the same pattern of expression, only its transcription decreases abruptly once aerial hyphae formation has begun, suggesting that RamC is dispensable once aerial hyphae formation is established [35•,36•]. If the only role for SapB were to act as a surfactant, then it would not be required once the aerial hyphae have extended into the air, and therefore, RamC would be superfluous once this has occurred.

One intriguing observation is that while the *ramR* mutant is unable to produce SapB, it is able to form a sparse aerial mycelium, relative to the wild type [34•,36•]. It is conceivable that there is some functional overlap between SapB and the chaplins (which have also been shown to have surfactant activity), and that the chaplins can partially substitute for the loss of SapB.

Conclusions

It is now apparent that microorganisms have evolved several distinct mechanisms by which to erect aerial filaments. A prevailing theme throughout, however, appears to be an important role for surface-active proteins at the air–water interface and on the surfaces of aerial structures. Fungal hydrophobins, often found in multiple copies, appear to have greater diversity in contributing to the development of fungal fruiting bodies than was previously envisaged, and coupled with this, is the recent finding that different morphogenetic proteins appear to be induced at different stages during aerial development [15•]. A similar situation appears to be emerging for the filamentous bacterium *Streptomyces coelicolor*, where three separate morphogenetic proteins

Figure 4



Model of morphogenetic protein activity in the formation of aerial hyphae in *S. coelicolor*. During vegetative growth **(a,b)**, SapB is secreted (blue dots) and assembles to form an amphiphilic sheet at the air-water interface (straight black line). This reduces surface tension and allows the emergence of aerial hyphae. Chaplin synthesis and secretion (red dots) begin during late vegetative growth **(b)**, and continue throughout aerial hyphal growth **(c,d)**. The chaplins polymerise to form a hydrophobic sheath surrounding the aerial filament, which further facilitates the growth into the air.

have been identified as having roles in aerial hyphae formation. These proteins appear to be expressed sequentially (SapB → chaplins → rodlins), and this likely reflects their predicted contribution to development. In our model (Figure 4), SapB is expressed first, and is secreted into the aqueous environment where it assembles at the air–aqueous interface to allow the emergence of aerial hyphae. This is aided by the action of the chaplins, which coat the aerial filaments and form a hydrophobic layer on the surface. Later, expression of the rodlins and possible rodlin–chaplin interaction would generate the rodlet ultrastructure that is characteristic of many *Streptomyces* spores. Stage-specific regulation of the genes that encode these morphogenetic proteins in the filamentous bacteria and fungi is therefore fundamental for aerial development.

So what are the key challenges for the future? In *S. coelicolor*, it seems likely that many of the key players in aerial development have now been identified, although the interactions between the chaplins, the rodlins, SapB and the bacterial cell surface are not well understood. Much work is also required to dissect the regulatory networks that coordinate the expression of these proteins, as there are still many unknowns. In filamentous fungi, hydrophobin-independent mechanisms for aerial development almost certainly exist, as evidenced by the existence of the repellents in *U. maydis* [13] and Sc15 in *S. commune* [15•]. These are, however, very specific examples, and there must be other surface-active morphogenetic proteins awaiting discovery. Finally, at some point in the future we must consider the oomycetes, which are mould-like in appearance but close taxonomic relatives of brown algae rather than fungi. Oomycetes provide virgin territory for the study of aerial development and it will be fascinating to determine whether evolution has selected for the presence of hydrophobin-like proteins in these unrelated, but morphologically identical eukaryotes or whether they have evolved a mechanism more similar to that used by *Streptomyces* to escape into the air.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Talbot NJ: **Coming up for air and sporulation**. *Nature* 1999, **398**:295-296.

2. Wösten HAB: **Hydrophobins: multipurpose proteins**. *Annu Rev Microbiol* 2001, **55**:625-646.

3. Kershaw MJ, Talbot NJ: **Hydrophobins and repellents: proteins with fundamental roles in fungal morphogenesis**. *Fungal Genet Biol* 1998, **23**:18-33.

4. Talbot NJ, Kershaw MJ, Wakley GE, DeVries OMH, Wessels JGH, Hamer JE: ***MPG1* encodes a fungal hydrophobin involved in surface interactions during infection-related development of *Magnaporthe grisea***. *Plant Cell* 1996, **8**:985-999.

5. •• Hakanpää J, Paananen A, Askolin S, Nakari-Setälä T, Parkkinen T, Penttilä M, Linder MB, Rouvinen J: **Atomic resolution structure of the HFBII hydrophobin, a self-assembling amphiphile**. *J Biol Chem* 2004, **279**:534-539.
Landmark report of the first structural characterization of a fungal hydrophobin. This revealed a compact globular structure built around four

intramolecular disulfide linkages and involving a previously undescribed protein fold.

6. Butko P, Buford JP, Goodwin SJ, Stroud PA, McCormick CL, Cannon GC: **Spectroscopic evidence for amyloid-like interfacial self-assembly of hydrophobin Sc3**. *Biochem Biophys Res Commun* 2001, **280**:212-215.

7. Mackay JP, Matthews JM, Winefield RD, Mackay LG, Haverkamp RG, Templeton MD: **The hydrophobin EAS is largely unstructured in solution and functions by forming amyloid-like structures**. *Structure* 2001, **9**:83-91.

8. • Whiteford JR, Lacroix H, Talbot NJ, Spanu PD: **Stage-specific cellular localisation of two hydrophobins during plant infection by the pathogenic fungus *Cladosporium fulvum***. *Fungal Genet Biol* 2004, **41**:624-634.
Distinct hydrophobins are produced at different stages of development including both invasive fungal growth and aerial development.

9. •• Fuchs U, Czymmek KJ, Sweigard JA: **Five hydrophobin genes in *Fusarium verticillioides* include two required for microconidial chain formation**. *Fungal Genet Biol* 2004, **41**:852-864.
An elegant study in which the roles of five hydrophobin genes in this corn pathogen are assessed. Reveals a role for hydrophobins in spore patterning, which highlights the distinct functions fulfilled by these surface-active proteins in fungal morphogenesis.

10. Lau GW, Hamer JE: ***Acropetal:* a genetic locus required for conidiophore architecture and pathogenicity in the rice blast fungus**. *Fungal Genet Biol* 1998, **24**:228-239.

11. • Paris S, Debeaupuis JP, Crameri R, Carey M, Charles F, Prevost MC, Schmitt C, Philippe B, Latgé JP: **Conidial hydrophobins of *Aspergillus fumigatus***. *Appl Environ Microbiol* 2003, **69**:1581-1588.
The conidial hydrophobin rodA is involved in resistance to attack by alveolar macrophages in this pulmonary pathogen.

12. • Mey G, Correia T, Oeser B, Kershaw MJ, Garre V, Arntz CC, Talbot NJ, Tudzynski P: **Structural and functional analysis of an oligomeric hydrophobin gene from *Claviceps purpurea***. *Mol Plant Pathol* 2003, **4**:31-41.
A pentameric hydrophobin comprising five discrete eight-cysteine hydrophobin domains is produced in the rye-grass pathogen, and secreted as a single large protein.

13. Wösten HAB, Bohlmann R, Eckerskom C, Lottspeich F, Bolker M, Kahmann R: **A novel class of small amphipathic peptides affect aerial growth and surface hydrophobicity in *Ustilago maydis***. *EMBO J* 1996, **15**:4274-4281.

14. Bohlmann R: **Isolierung und charakterisierung von filamentspezifisch exprimierten genen aus *Ustilago maydis***. Thesis, Fakultätfür Biologie, Ludwig-Maximilian-Universität, München, 1996.

15. • Lugones LG, de Jon JF, de Vries OMH, Jaiving R, Dijkterhuis J, Wösten HAB: **The SC15 protein of *Schizophyllum commune* mediates formation of aerial hyphae and attachment in the absence of the SC3 hydrophobin**. *Mol Microbiol* 2004, **53**:707-716.
A non-hydrophobin dependent mechanism for aerial development may be present in *Schizophyllum commune*, explaining why *Δsc3* deletion mutants can still make some aerial hyphae.

16. •• Claessen D, Wösten HAB, van Keulen G, Faber OG, Alves AMCR, Meijer WG, Dijkhuizen L: **Two novel homologous proteins of *Streptomyces coelicolor* and *Streptomyces lividans* are involved in the formation of the rodlet layer and mediate attachment to a hydrophobic surface**. *Mol Microbiol* 2002, **44**:1483-1492.
The formation of the characteristic 'rodlet' pattern seen on the surface of spores is dependent on two homologous proteins, RdlA and RdlB, which are thought to aid in the attachment of hyphae and spores to hydrophobic surfaces.

17. • Claessen D, Stokroos I, Deelstra HJ, Penninga NA, Bormann C, Salas JA, Dijkhuizen L, Wösten HAB: **The formation of the rodlet layer of streptomycetes is the result of the interplay between the rodlins and chaplins**. *Mol Microbiol* 2004, **53**:433-443.
Two homologous proteins, RdlA and RdlB, contribute to the formation of the rodlet layer on the surface of spores, possibly in a chaplin-dependent manner.

18. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S: **Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis***. *Nat Biotechnol* 2003, **21**:526-531.

19. Lauter FR, Russo VE, Yanofsky C: **Developmental and light regulation of *eas*, the structural gene for the rodlet protein of *Neurospora***. *Genes Dev* 1992, **6**:2373-2381.

20. Wösten HA, Schuren FH, Wessels JG: **Interfacial self-assembly of a hydrophobin into an amphipathis protein membrane mediates fungal attachment to hydrophobic surfaces**. *EMBO J* 1994, **13**:5848-5854.

21. •• Claessen D, Rink R, de Jong W, Sieebring J, de Vreugd P, Boersma FGH, Dijkhuizen L, Wösten HAB: **A novel class of secreted hydrophobic proteins is involved in aerial hyphae formation in *Streptomyces coelicolor* by forming amyloid-like fibrils**. *Genes Dev* 2003, **17**:1714-1726.
The chaplin proteins were purified from the surface of the spores and were identified by N-terminal sequencing. They are important for aerial hyphae formation, and appear to be capable of acting as surfactants. *In vitro*, the chaplins can polymerise to form amyloid-like fibres.

22. •• Elliot MA, Karoonuthaisiri N, Huang J, Bibb MJ, Cohen SN, Kao CM, Buttner MJ: **The chaplins: a family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor***. *Genes Dev* 2003, **17**:1727-1740.
Using microarray analysis, eight chaplin genes were identified as being down-regulated in a mutant unable to raise aerial hyphae (*bldN*), relative to the wild type strain. This pattern of expression was seen in all *bld* mutants examined. The chaplin proteins are localized to the cell wall and are essential for the formation of aerial hyphae.

23. Mazmanian SK, Liu G, Ton-That H, Schneewind O: ***Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall**. *Science* 1999, **285**:760-763.

24. Elliot M, Damji F, Passantino R, Chater K, Leskiw B: **The *bldD* gene of *Streptomyces coelicolor* A3(2): a regulatory gene involved in morphogenesis and antibiotic production**. *J Bacteriol* 1998, **180**:1549-1555.

25. Elliot MA, Bibb MJ, Buttner MJ, Leskiw BK: **BldD is a direct regulator of key developmental genes in *Streptomyces coelicolor* A3(2)**. *Mol Microbiol* 2001, **40**:257-269.

26. Bibb MJ, Molle V, Buttner MJ: **σ^{BldN}, an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2)**. *J Bacteriol* 2000, **182**:4606-4616.

27. Takano E, Tao M, Long F, Bibb MJ, Wang L, Li W, Buttner MJ, Bibb MJ, Deng ZX, Chater KF: **A rare leucine codon in *adpA* is implicated in the morphological defect of *bldA* mutants of *Streptomyces coelicolor***. *Mol Microbiol* 2003, **50**:475-486.

28. Nguyen KT, Tenor J, Stettler H, Nguyen LT, Nguyen LD, Thompson CJ: **Colonial differentiation in *Streptomyces coelicolor* depends on translation of a specific codon within the *adpA* gene**. *J Bacteriol* 2003, **185**:7291-7296.

29. Guijarro J, Santamaria R, Schauer A, Losick R: **Promoter determining the timing and spatial localization of transcription of a cloned *Streptomyces coelicolor* gene encoding a spore-associated polypeptide**. *J Bacteriol* 1988, **170**:1895-1901.

30. Tillotson RD, Wösten HAB, Richter M, Willey JM: **A surface active protein involved in aerial hyphae formation in the filamentous fungus *Schizophillum commune* restores the capacity of a bald mutant of the filamentous bacterium *Streptomyces coelicolor* to erect aerial structures**. *Mol Microbiol* 1998, **30**:595-602.

31. Willey J, Santamaria R, Guijarro J, Geistlich M, Losick R: **Extracellular complementation of a developmental mutation implicates a small sporulation protein in aerial mycelium formation by *S. coelicolor***. *Cell* 1991, **65**:641-650.

32. Ma H, Kendall K: **Cloning and analysis of a gene cluster from *Streptomyces coelicolor* that causes accelerated aerial mycelium formation in *Streptomyces lividans***. *J Bacteriol* 1994, **176**:3800-3811.

33. Ueda K, Miyake K, Horinouchi S, Beppu T: **A gene cluster involved in aerial mycelium formation in *Streptomyces griseus* encodes proteins similar to the response regulators of two-component regulatory systems and membrane translocators**. *J Bacteriol* 1993, **175**:2006-2016.

34. • Nguyen KT, Willey JM, Nguyen LD, Nguyen LT, Viollier PH, Thompson CJ: **A central regulator of morphological differentiation in the multicellular bacterium *Streptomyces coelicolor***. *Mol Microbiol* 2002, **46**:1223-1238.
bld mutant strains are unable to raise aerial hyphae and are unable to produce the morphogenetic peptide SapB, but overexpression of the *ram* gene cluster, and the *ramR* gene alone, could restore SapB production and aerial hyphae formation to all *bld* mutants tested. RamR controls the expression of *ramC*, and disruption of *ramR* abolishes SapB production.

35. • Keijser BJF, van Wezel GP, Canters GW, Vijgenboom E: **Developmental regulation of the *Streptomyces lividans ram* genes: involvement of RamR in regulation of the *ramCSAB* operon**. *J Bacteriol* 2002, **184**:4420-4429.
Transcriptional analysis of the *ram* cluster reveals *ramCSAB* to be an operon whose expression requires growth on rich media and several *bld* genes.

36. • O'Connor TJ, Kanellis P, Nodwell JR: **The *ramC* gene is required for morphogenesis in *Streptomyces coelicolor* and expressed in a cell type-specific manner under the direct control of RamR**. *Mol Microbiol* 2002, **45**:45-57.
Disruption of *ramC* and *ramR* reveal that both genes are necessary for the formation of aerial hyphae. The regulation of *ramC* transcription is complex: it is dependent on *bldD* and *cprA*, independent of *bldM* and *bldN*, directly controlled by RamR, and spatially constrained to the substrate hyphae.

37. Hudson ME, Zhang D, Nodwell JR: **Membrane association and kinase-like motifs of the RamC protein of *Streptomyces coelicolor***. *J Bacteriol* 2002, **184**:4920-4924.

38. •• Kodani S, Hudson ME, Durrant MC, Buttner MJ, Nodwell JR, Willey JM: **The SapB morphogen is a lantibiotic-like peptide derived from the product of the developmental gene *ramS* in *Streptomyces coelicolor***. *Proc Natl Acad Sci USA* 2004, **101**:11448-11453.
The morphogenetic peptide SapB is produced via the activity of the *ramCSAB* gene cluster. RamC appears to direct the processing of the C-terminal 21 amino acids of RamS, forming a lantibiotic-like peptide that is SapB.

39. Saul H-G, Bierbaum G: **Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria**. *Annu Rev Microbiol* 1998, **52**:41-79.

40. Ueda K, Oinuma K, Ikeda G, Hosono K, Ohnishi Y, Horinouchi S, Beppu T: **AmfS, an extracellular peptidic morphogen in *Streptomyces griseus***. *J Bacteriol* 2002, **184**:1488-1492.

41. Willey J, Schwedock J, Losick R: **Multiple extracellular signals govern the production of a morphogenetic protein involved in aerial mycelium formation by *Streptomyces coelicolor***. *Genes Dev* 1993, **7**:895-903.